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# Testing of Antibacterial Activity of Ethanol Extracts of Papuan Herbs as a *Chemical Library* of the Bacterium *Salmonella typhimurium*

**Rosye H. R. Tanjung,[1] and Yohanis Ngili[2]***

**[1] Department of Biology, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura-Indonesia**
**[2] Biochemistry Research Group, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura-Indonesia**

**Abstract :** Diversity of plants in Indonesia as *Chemical library*, has long been used as a medicine. Interaction of chemical compounds to a target molecule is one step in a series of studies for drug discovery. This research was conducted in order to determine whether there is an interaction between ethanol extracts of herbs with *Salmonella typhimurium* bacteria (that cause disease 'like typhoid' in mice), both at the cell and molecular level. Previous research has been able to demonstrate their interaction with DNA ethanol extracts of herbs *Salmonella typhimurium* through HPLC method. In this study the interaction is not solely between the ethanol extract of herbs and DNA alone but more thorough ie the components of bacterial cells *Salmonell typhimurium*. To achieve these objectives have been carried interaction at the cellular level by looking at the growth of bacteria on a petri dish; and molecular level by way of lysis of the bacteria. Observations at the cellular level, carried out by culturing the bacteria on solid media herbs that have various concentrations and volume variations, the analysis shows the growth of bacteria in all media. Bacteria growth on media have visitors showed interaction at the cellular level is not observed as previous research. The interaction at the molecular level that has been done by way of bacterial lysis, followed by immobilization on the membrane components with the aid of UV light and then incubated in the ethanol extract of herbs, showed a peak of HPLC chromatogram of ethanol extracts of herbs are missing. Their peak chromatograms with Rf 0.931 were missing in the second experiment gives a strong presumption that there is interaction between herbal extracts and molecules *Salmonella typhimurium* bacteria. Based on this research can be concluded that the ethanol extract of herbs not only interact with DNA, but also with other molecules *Salmonella typhimurium.*
**Keywords**: Ethanol extracts of herbs, *Salmonella typhimurium*, antibacterial activity, chemical library, and HPLC analysis.

## Introduction

The results of the research that has been developed and will be developed in the area of new drug discovery is currently growing in line with the growing need for drugs. Drug discovery begins with finding a compound that has a specific biological activity. To achieve this, research is usually performed with encounters a collection of chemical compounds (*chemical library*) on a selected target molecules and eventually obtained a *chemical library* component in the that has a specific activity.[1-2] Today arose a new phenomenon to combine the two major forces in front of us is the source of wealth of biodiversity that exists in nature Indonesia, especially

in the provinces of Papua and research results world genomes of both the human genome, plant genome, and the genome of the microorganism. Biological natural resources can be used as a *chemical library* that will be obtained by a component of the natural resources that can be used in drug development. Sources of biological nature can be exploited through a simple chemical process that is extraction. The use of the wealth of natural materials is very advantageous considering Indonesia is naturally blessed with diverse natural resources.[3]

To investigate whether there is any interaction between the target molecule with *chemical library* screening methods normally used. One of the screening methods used in drug discovery research is the *High Throughput Screening* (HTS). HTS is an instrument with cutting edge technology that can detect the molecular level interactions between a chemical compound with the target molecule. Methods of screening using this tool can be done on a large scale because of the HTS has the advantage to screen at very high speeds, which can detect a sample of up to 1,000 samples per week even more. However, this HTS methods also have disadvantages, namely a long process and thus also takes a long time to arrive at the stage of finding compounds that interact and also the amount spent enormous.

Based on the existing weaknesses, this study aims to find an alternative method of detecting interaction between a chemical compound with a target molecule. The development of this method is expected to be generating a more effective method by phasing procedure simpler.[4-6] As a chemical library provides a collection of chemical compounds that will be used to detect interactions traditional Indonesian herbal medicine. Selection of herbal medicine as a *chemical library* for herbal medicine itself is a result of extraction of natural wealth of Indonesia and has been known to useful by those who use it. Wealth of natural materials when utilized optimally will contribute significantly to human interests. Moreover, because herbal medicine has been used as a drug it is predicted that these herbs contain components that have a specific activity that can be detected with this alternative method.

In this study the DNA of the bacteria *Salmonella typhimurium* is used as a model for the target molecule. *S. typhimurium* bacteria in general are known to be harmful organisms that cause typhoid fever in mice. Besides S. typhimurium is also a type of bacteria that is cultured in laboratory conditions until will facilitate the research process. The amount of research on this bacterium *S. typhimurium* made into an organism that is most understandable. Another reason is that it is often used as a model in the drug discovery process as *S. typhimurium* have DNA similar to human DNA.

## Material and Methods

### Culturing of the bacterium *Salmonella typhimurium*

Planting bacteria on solid LB media performed in sterile conditions. Therefore, to avoid the presence of contaminants that get into the media, especially media poured into Petri, the *laminar air flow* sprayed by the aqueous ethanol solution and work near the fire. The bacteria are grown in LB medium (1% tryptone, 1.5 bakto agar, 1% NaCl, and 0.5% yeast extract in 100 mL of distilled water), so that the sterile solution is autoclaved. 100 mL of this medium can be made to 5 petri dish. After autoclaved solution is cooled since the temperature in the autoclave can reach 100 °C. After a rather cool but not freeze up, the solution is poured into a petri. Petri dishes containing agar medium was allowed for 24 h to ensure no bacterial growth due to contamination of the environment. If there is no contamination of the media that is ready for use. Planting bacteria is done by scraping bacteria seeds (*glycerol stock*) with the stem loop or spread on the surface of a solid medium, the work must be performed in a laminar flow. Once the bacteria are grown in media, petri dish was closed and re-wrapped with paper. Furthermore incubated for 14-17 h in an incubator having a temperature of 37 °C, after which the bacteria grow, the media are removed from the incubator, and stored at 4 °C so that bacteria do not continue to grow but not dead. Stock bacteria can be used within a period of one month.[7-10]

### Making ethanol extracts of herbs with soxhlet

Materials used consist of as many as 50 grams of herbal medicine and ethanol p.a. The herbal medicine is wrapped tightly as possible with paper filter, so that herbs are not out of the package then before being put into Soxhlet, approximately 200 mL of ethanol p.a. put into it, and then extracted at 50 °C to 7 times. This process takes approximately 6-7 h. The ethanol extract is obtained, put in Erlenmeyer and covered with aluminum foil and stored at 4 °C in the refrigerator as a stock.

**Interactions at the cellular level between the bacterium *Salmonella typhimurium* and ethanol extract**

Making media containing herbs is equal to the growth media, after the solution was put into a petri dish media, while not yet frozen, the ethanol extract of herbs added with a micropipette and then stir until evenly distributed. Solid LB media have visitors is made with various concentrations of 25%, 50%, 60%, 80%, and the variation of the volume of 50, 100, 200. 300, and 400 mL.

**Lysis of cells and their interaction with ethanol extraction**

In this research, cell lysis is done by first doing blot bacteria on *Hybond* $N^+$ nylon membrane. Pemblotan performed with a silica bacteria on *Hybond* $N^+$ nylon membrane (Amersham). The bacteria used can be derived from glycerol stocks or stock order.

Lysis process was conducted by a nylon membrane *Hybond* $N^+$ in the above collision (3 layers) *Whatman* filter paper that has been saturated with lysis solution. The process of cell lysis in this way consists of three phases, the first Hybond N + membrane that has doted bacteria placed on Whatman filter paper saturated with a solution of 10% SDS. This solution was allowed to permeate through the membrane pores and damage the cell wall of bacterial cells without changing the position for 3 min, then moved to the second stage membrane on Whatman others who have been saturated by a solution of NaOH 0.5 M and 1.5 NaCl for 5 min and the third phase of the membrane is moved onto Whatman others who have been saturated with a solution of 1.5 M NaCl, 0.5 M Tris-Cl pH 8 for 5 min.[7]

After lysis is complete membrane lysis along with cells that had been dried at room temperature for 30-60 min. All of the above work to be done in a laminar flow, the next in order to keep the glue on the lysis nylon membrane, the membrane is irradiated with UV for 1 min. After it was prepared 3 eppendorf tube containing 1.5 mL of ethanol extracts of herbs that had been diluted 100 times. The first tube contained only herbal extracts, the second tube by membrane without the bacteria that have been exposed to UV and the third is filled membrane which contains cells that have lysis doted. All three tubes were incubated in shaking incubator at 37 °C for 18 h.

**The process of analysis by thin layer chromatography**

Analysis by thin layer chromatography performed with a few variations of the eluent. Variations eluent used was methanol : water with a ratio of 6:4 (w/v) hexane : ethyl acetate in a ratio of 1:1 (w/v), chloroform : methanol with a ratio of 9:1 (w/v), and Last chloroform : methanol in the ratio 1:1 (w/v). For the purposes of this thin layer chromatography prepared silky gel thin plate that cut the size of 9x1 cm. Then given upper and lower limits so that the course of eluent as high as 8 cm. In the silica plate sample spotted ethanol extracts of herbs after interaction with DNA and also standardized herbal extracts ethanol without any interaction with DNA. The plates are then entered to the eluent in the chamber and wait till the eluent rises to a specified threshold. Same process is done for each variation eluent.

**The process of analysis by high performance liquid chromatography**

The process of analysis by HPLC is quite simple: by using HPLC column type used are $C_{18}$ columns with a length of 15 cm. Wavelength measurement time is 254 nm and the flow rate was adjusted to 1 mL/min.[7]

Eluent that are used in this analysis is a variation of eluent methanol : water with a ratio of 6:4 (v/v). Before use eluent is inserted first into the ultrasonic bath to improve the quality of the eluent them to precipitate impurities that may be present in the eluent. The analysis was performed by injecting samples of the ethanol extract of herbs on HPLC instrument. Then from the HPLC apparatus will be printed chromatogram of the separation of the sample is injected. The same thing has been done to a standard herbal extracts without any interaction with DNA in order to obtain two chromatograms, standards and samples, which will then be compared to analyze the interactions happened.

## Results and Discussion

### Salmonella typhimurium bacterial cultures on solid media that contain herbs

In this study, the interaction observed through the results of *Salmonella typhimurium* bacteria cultivation on solid media that contain herbs, levels of ethanol extract of herbs given to the media in a variety of concentrations and volumes began to lower levels, till fairly extreme levels.[11] Before the ethanol extract was diluted to obtain various concentrations, ethanol made herbal extracts by using soxhlet. Results of the extraction and concentration is considered to be a stock of 100%. Variations in the concentration and volume can be seen in the table below along with the observation of bacterial culture (Table 1).

**Table 1. Results planting bacteria in media containing herbs, mark (√) showed bacteria grow in every medium with varying concentrations and volumes that have been tested, the sign (-) experimental variations in the concentration and volume that are not carried**

| Concentration (%) | Volume of ethanol extract of herbs (µL) | | | | |
|---|---|---|---|---|---|
| | 50 | 100 | 200 | 300 | 400 |
| 25 | √ | - | √ | - | - |
| 50 | - | - | √ | - | - |
| 60 | √ | √ | √ | √ | - |
| 80 | √ | √ | √ | √ | √ |

The presence of bacteria growing on solid media seen with the white dots are coincident and follow certain paths in accordance with the way we pull the rod ose used. The dots are bacteria colonies where each colony consists of thousands of bacterial cells. In this study, not all variations in the concentration or the volume performed, but as we see in the table above. Each experiment with variations in the concentration and volume is done showed the presence of bacteria that grow. While variations in the concentration and volume carried by dashes.

If we observe the result, at low concentrations of 25%, 50 µL, bacteria are still able to grow even in the extreme conditions of 80%, 400 µL of the bacteria are still able to reproduce well. This suggests that the interaction at the cellular level has been observed. Basically foreign chemical substances are toxic at certain levels. Moreover, given excessive doses, it can cause poor growth can even be lethal (causing death). This study proves the bacteria continued to grow on an agar medium that has been given chemical compound. In this case the compounds that are components of the ethanol extract of herbs. But that does not mean there is no interaction at all because this way we can not determine whether the bacteria that grows normally like without herbs or decline in growth because we do not count the number of bacteria are grown and the number of bacteria after proliferate within a certain time.

Nevertheless the possibility that the bacteria do not interact at the cellular level remain. It can be caused by the inability of the ethanol extract of herbs diffuse component dinsing penetrate cells. Therefore, the research continued with the ethanol extract of herbs encounters with component cell lysis and observed through a standard chromatogram HPLC and sample results.

### Interaction ethanol extract with the components of bacterial cell lysis *Salmonella typhimurium*

In this study the interaction between ethanol extracts of herbs with the bacterium *Salmonella typhimurium* which has undergone lysis. Thus the possibility of interactions that occur there will be more because not only DNA but with other components present in the cell.

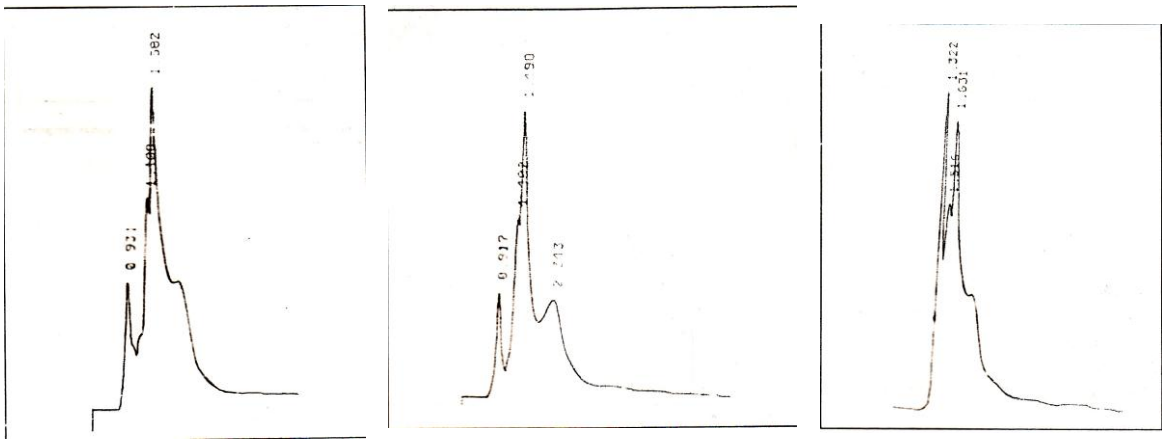
Lysis of bacteria performed in this study adopts partially blotting method of DNA hybridization of bacterial colonies, without isolation of DNA. Lysis process performed by letting the lysis solution is absorbed by the filter paper. Then the solution penetrates the pores of the membrane of nylon *Hybond* $N^+$ and ripped off the wall and cell membrane. In this way, the position of bacterial cell membranes unchanged.

SDS lysis solution first is 10%. SDS is a surface active agent or surfactant, because it has a hydrophobic part, this section will go into the membrane and ruin because the membrane consists of two layers of lipid bilayer consisting of more nonpolar. Besides the surfactant causes the pressure difference between the inside of the cell and the outside environment (a solution hipotonis), as a result of water into the cells so that the cells swell and rupture. NaOH and NaCl are compounds which can denature the protein because it can absorb water for solvation process of (veil) protein, so as to facilitate the interaction with chemical compounds that are components of ethanol extracts of herbs. The lysis of cells that have been neutralized again with a solution of NaCl, Tris-Cl, and buffer pH 8.

Lysis of cells attached to the membrane is then dried and irradiated with UV in order lysis was attached to the membrane. Irradiation causes the membrane to become active binding lysis, so it will not mix with the ethanol extract that interact with it. The components of the ethanol extract of herbs that interact will be bound to the membrane or cell lysis. To observe whether there is such interaction, then analyzed by HPLC.

## Results of HPLC analysis

HPLC analysis performed on three samples, namely: ethanol extracts of herbs that have been incubated to obtain a standard chromatogram, the ethanol extract of herbs that interact with mambran and ethanol extracts of herbs that interact with the membrane attached cell lysis. Standard chromatogram showing the number of components in the ethanol extracts of herbs and their composition (**Fig. 1.a**). The second chromatogram have peak shape similar to the first chromatogram (**Fig. 1.b**), and the third chromatogram (**Fig. 1.c**) showed loss of the first peak contained in the standard chromatogram.



**A** **B** **C**

**Fig. 1. Chromatogram. (A) the standard chromatograms of ethanol extract of herbs without membrane or cell lysis, which was incubated at 37 °C; (B) one sample chromatogram, the chromatogram produced from the ethanol extract of herbs that have been given membrane; and (C) sample chromatogram 2, the chromatograms of ethanol extract of herbs that have interacted with membrane or cell lysis of bacteria that have been incubated at 37 °C.**

From the three images could be evidenced the ethanol extract herbal interactions with components in bacteria. The loss of the chromatogram peaks at Rf 0.931 shown in Fig. 1.c is evidence of an interaction between ethanol extracts of herbs with the components of bacterial cells for possible interactions between chemical compounds extracted with membranes did not take place, evidenced by the absence of the missing peaks on the chromatogram sample 1.

The results of the chromatogram can be explained using a model example of a chromatogram. For example, there are five components of chemical compounds from herbs extracted by ethanol, there will be five peaks in standard chromatograms of ethanol extract of herbs such as in Fig. 2, assuming the component is a component of A, B, C, D, and E.

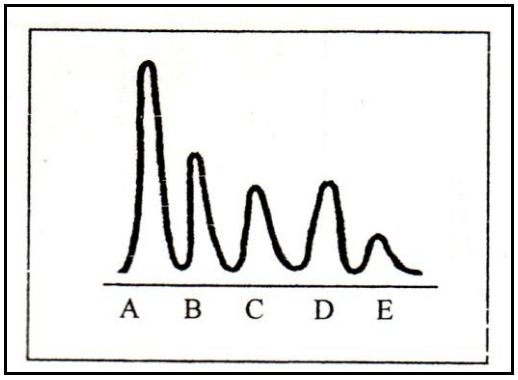



**Fig 2. Model standard chromatogram, if there are five compounds were extracted by ethanol it would appear five peaks in the chromatogram**

Possibility of interactions that occur are as follows: one of the components such as A, interact with the membrane alone; A component interacts with the bacteria; A component interacts with both the bacteria and the membrane. Another possibility is more than one component, for example A and B, which interacts with the membrane alone, bacteria, or both, as stated previously.

Possibility is where one component is a membrane to interact with it, then the chromatogram obtained is shown in Fig. 3.

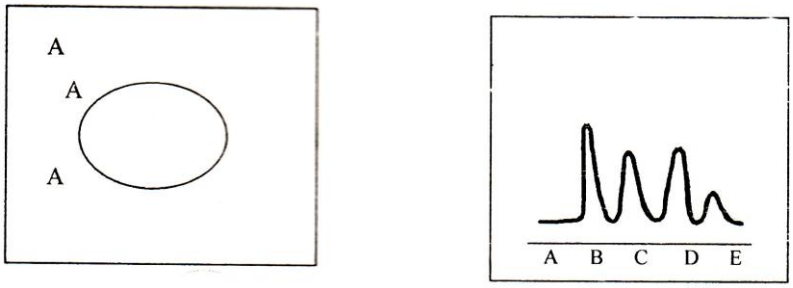



**Fig 3. Model of chromatogram, which would be obtained if the extracted chemical compounds interact with the membrane alone**.

Basically, the interaction with the membrane not only can be demonstrated by the loss of one chromatogram peak but can be also shown by the reduction of the peak. This can happen if the component has a sizeable amount while the membrane surface is limited or impeded by bacteria. It can also occur with the interactions with bacteria and other research that related with mutation the human DNA or mtDNA of organisms.[11-20]

## Conclusion

At the cellular level of interaction is done by planting bacteria in media containing medicinal herbs to vary the concentration, with small concentrations of up to a high enough concentration to determine the possibility of no growth of bacteria at these concentrations. Herbal extraction is done by using soxhlet order to extract obtained more concentrated, but the experimental results of interaction at the cellular level has yet to be observed for the bacteria continued to grow in all media despite the concentration of ethanol extracts of herbs is high. At the molecular level interactions observed are between molecules herbs with all components in bacteria (not only by DNA alone) after the previous lysis of the bacteria by blotting on the membrane and then immobilized with irradiated UV subsequently inserted into the tube containing the ethanol extract of herbs. To detect and view their interactions at the molecular level, the ethanol extract of the herbal medicine was observed by means of HPLC. Observation of HPLC chromatogram prove their interactions at the molecular level as indicated by the loss of sample chromatogram peak at Rf 0.931. Thus it can be concluded that at the molecular

level interaction occurs between the ethanol extract of herbs with all the components of bacterial cells and not just DNA alone.

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